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Effects of Age and Obesity on the Metabolism of Lindane by Black a/a, Yellow (A sup vy)/a, and Pseudoagouti (A sup vy)/a Phenotypes of (YS x VY) F1 Hybrid Mice

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16. ABSTRACT

Lindane (γ-hexachlorocyclohexane) has been shown to produce hepatomas in some strains of mice but not in others. Genetic factors and/or altered metabolism may plan a role in the susceptibility to lindane-induced hepatomas. Previous investigations have demonstrated that the viable yellow A^{vy} mutation increases susceptibility to the formation of hepatomas in the mouse. This study reports the effect of age and obesity on the comparative metabolism and disposition of lindane in obese yellow A^{vy}/a and in lean pseudoagouti A^{vy}/a and black a/a phenotypes of (YS x VY) F-1 hybrid female mice at 8, 17, 30, 56, and 86 weeks of age. Twenty-four hours prior to sacrifice the mice were dosed p.o. with 18 mg lindane (containing 55 μCi [U-^{14}C] lindane)/kg. Aging altered the biotransformation of lindane such that while the excretion of lindane and its metabolites declined, the proportion of conjugated and polar metabolites were elevated. Tissue storage was elevated in older animals. Accelerated and prolonged growth, reduced metabolite excretion, a greater proportion of conjugates and higher dechlorinase activity associated with obesity suggest possible explanations for enhanced susceptibility of the yellow A^{vy}/a mice to lindane-induced hepatomas as compared with the pseudoagouti A^{vy}/a and black a/a siblings.

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EFFECTS OF AGE AND OBESITY ON THE METABOLISM OF LINDANE BY BLACK *a/a*, YELLOW A^{vy}/a, AND PSEUDOAGOUTI A^{vy}/a PHENOTYPES OF (YS × VY) F_1 HYBRID MICE

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INTRODUCTION

Lindane (γ-hexachlorocyclohexane), an organochlorine insecticide that has been employed in pharmaceutical preparations, on livestock, and in various agricultural applications, has been repeatedly tested for carcinogenic activity in rodents (Fitzhugh et al., 1950; Ortega et al., 1957; Goto et al., 1972; Nagasaki et al., 1972; Didenko et al., 1973; Hanada et al., 1973; Ito et al., 1973, 1975; Thorpe and Walker, 1973; Herbst et al., 1975; National Cancer Institute, 1977). In three of these studies, which employed mice, oncogenic change, based on the appearance of liver tumors, was reported (Goto et al., 1972; Hanada et al., 1973; Thorpe and Walker, 1973).

Since most chemical carcinogens in the environment require metabolic activation (Miller and Miller, 1974), the difference in susceptibility of various strains of mice to lindane-induced hepatomas could be due to differences in the biotransformation of the pesticide (Robinson et al., 1975; Thorgeirsson and Wirth, 1979). Both aging and lindane treatment can influence metabolism. Lindane induces hepatic microsomal enzyme activity (Kolmodin-Hedman et al., 1971; Chadwick et al., 1971; Den Tonkelaar and Van Esch, 1974; Mikol et al., 1980) and, upon repeated administration, stimulates its own metabolism (Chadwick and Freal, 1972). In addition, aging has also been shown to influence hepatic enzyme activity (Kato and Takanaka, 1968a,b; Chadwick et al., 1975a; Schmucker and Wang, 1980; Rikans and Notley, 1982) as well as susceptibility to hepatotoxic injury (Rikans, 1984).

The mechanisms by which genetic factors modify the spontaneous and induced incidence of mouse hepatomas are not understood. However, the most suggestive evidence for involvement of a specific gene locus in enhancing formation of mouse hepatomas is the influence of the A^y (lethal yellow) and A^{vy} (viable yellow) genes at the agouti locus on the incidence of hepatomas in inbred C3H mice and their F_1 hybrids (Heston and Vlahakis, 1961, 1966, 1968). Recently it was reported that lindane induced significantly more liver tumors in yellow A^{vy}/a female mice than in either their pseudoagouti A^{vy}/a or black a/a sisters (Wolff, 1985).

Whether increased hepatoma formation is a direct effect of these genotypes or an indirect one mediated by the obesity of the yellow mice has been uncertain. To clarify this point, the effect of age, obesity, and chronic lindane treatment on the metabolism of lindane by female (YS × VY) F_1 hybrid mice and the induction of hepatic neoplasms in these mice was studied as part of a collaborative effort with the National Center for Toxicological Research (NCTR). The influence of obesity and increasing age on the comparative biotransformation of lindane by yellow A^{vy}/a, pseudoagouti A^{vy}/a, and black a/a (YS × VY) F_1 hybrid mice is reported here. The effects of chronic lindane treatment

on lindane metabolism and hepatoma formation in obese and lean mice will be reported in separate papers.

METHODS

Animals

Mice used in this study were either isogeneic or differed only by one allele at the agouti locus on chromosome 2. A cross of YS/ChWffC3Hf/Nctr-*a/a* females × VY/WffC3Hf/Nctr-A^{vy}/a males (Lyon, 1981) was used to produce F_1 hybrids (Wolff, 1978) as follows:

Genome	Genotype	Phenotype
(YS × VY) F_1	A^{vy}/a	Yellow (obese)
(YS × VY) F_1	A^{vy}/a	Pseudoagouti (lean)
(YS × VY) F_1	*a/a*	Black (lean)

The A^{vy}/a embryos differentiate into two phenotypic classes, mottled yellow (hereafter called yellow) and pseudoagouti. The yellow A^{vy}/a mice become fat, while the pseudoagouti A^{vy}/a mice remain lean. The proportion of A^{vy}/a zygotes that differentiates into each of these phenotypic classes is determined to a major extent by local microenvironmental conditions in the dam's reproductive tract that are associated with the dam's strain genome (Wolff, 1978). The expression of the A^{vy}/a genotype in two distinct phenotypic classes with very different metabolic dynamics provides a means of separating phenotypic from genotypic effects of the A^{vy}/a gene (Roberts et al., 1984).

One of the objectives of this work was the detection of lindane-induced hepatoma formation. Thus low spontaneous hepatoma formation in the test animal is desirable. To this end, female mice, which are less susceptible to spontaneous hepatoma formation than males, were employed (Frith et al., 1983; Reuber, 1979). Moreover, inbred YS/ChWF and Y/Wf, the parent strains of the mice used in the present study, have a very low spontaneous incidence (Wolff and Pitot, 1972). At 12–16 mo of age, yellow A^{vy}/a and black *a/a* males of the inbred YS strain had an 11% and 3% spontaneous incidence of hepatomas, respectively, while at the same age yellow A^{vy}/a and black *a/a* males of the inbred VY strain had spontaneous incidences of 24% and 13%, respectively.

Experimental Design

At 4, 13, 26, 52, and 82 wk of age, 18 obese yellow, 18 lean pseudoagouti, and 18 lean black (YS × VY) F_1 hybrid female mice from the specific pathogen-free NCTR breeding colony together with a supply of autoclaved Purina 5010M chow (4.3–6.7% fat) were shipped from NCTR

by air to the Environmental Protection Agency Environmental Research Center (EPA ERC), Research Triangle Park, N.C. These ages were selected to span the period between the beginning of treatment and the age at which lindane-induced tumor formation was anticipated. During a 4-wk acclimation period at EPA ERC, the mice, housed three per cage in polycarbonate cages covered with spun-polyester filter bonnets and equipped with Hunziker feeders, were maintained on the Purina chow. The animals were placed in a Bioclean class 100 laminar flow clean room (Hazleton Systems, Aberdeen, Md.) provided with high-efficiency particle-filtered air at a rate of 15 changes/h at 22 ± 2°C and 50 ± 5% relative humidity. A photoperiod, without twilight, provided 12 h of daylight commencing at 0700 h.

Tissue Storage and Metabolism

At 24 h prior to necropsy the animals were dosed p.o. with 18 mg lindane (containing 55 μCi [U-^{14}C]lindane/kg) in a volume of peanut oil equivalent to 0.25% of the body weight. The [U-^{14}C]lindane (specific activity 82 mCi/mmol, radiochemical purity 97%) was obtained from Amersham, Arlington Heights, Ill. The unlabeled lindane (>99% γ-hexachlorocyclohexane) was obtained from the EPA Health Effects Research Laboratory Analytical Reference Standards Repository, Research Triangle Park, N.C. Preliminary work indicated that this dose was well tolerated by the (YS × VY) F_1 hybrid mice and permitted detection of all the lindane metabolites. After treatment with [U-^{14}C]lindane, the mice were transferred, three to a cage, to animal containment chambers (Plas-Labs, Lansing, Mich.), and urine, feces and expired air were collected for 24 h. At necropsy, samples of urine, feces, expired air, liver, kidney, blood, and adipose tissue were taken for analysis of radioactivity.

While urine and expired air extracts were counted directly, liver, kidney, fat, blood, and feces samples were subjected to combustion in a Packard Tri-Carb model 306 sample oxidizer and the radioactivity was determined in a Packard Tri-Carb model 3380 liquid scintillation spectrometer employing a quench correction curve. Hepatic enzyme activities involved in the dehydrogenation of lindane and the dechlorination of lindane were determined (Chadwick et al., 1975b). Lipids were extracted using a modification (Hanson and Olley, 1963) of the Bligh and Dyer method (Bligh and Dyer, 1959). They were assayed by the method of Fewster et al. (1969), modified as follows: thin-layer chromatographic plates were sprayed with 6% cupric acetate in 15% phosphoric acid and were heated at 160°C for 15 min.

Urine samples were extracted, and the extracts were analyzed on a Tracor MT-220 gas chromatograph equipped with an electron-capture detector as previously described and modified (Chadwick et al., 1977, 1978a,b).

Statistical Analysis

Duncan's multiple-range test (Duncan, 1955) and analysis of variance procedures (SAS Institute Statistical Analysis System, 1982) were used as aids in the interpretation of the data from this study. Bartlett's test was used to assess homogeneity of variance (Anderson and McLean, 1974). Where heterogeneity was encountered, the data were suitably transformed prior to analysis. Differences were considered significant at $p < 0.05$. The p values in this study should not be taken as confirmatory hypothesis testing significance levels but rather as directional indicators.

RESULTS

The obese yellow *A^vy/a* mice attained significantly greater body weight, liver weight, and kidney weight than either the pseudoagouti *A^vy/a* or the black *a/a* mice by 30 wk of age (Fig. 1).

From 8 to 86 wk of age, the yellow mice exhibited a marked 60% greater increase in body weight, a 20–30% greater increase in kidney weight, and a 10–20% greater increase in liver weight than their nonyellow siblings.

Significant elevation in the organ and body weight of the yellow mice also influenced the tissue storage of lindane-derived radioactivity in these animals (Table 1). In addition to their greater body and organ weight, the yellow mice have a markedly greater capacity for storage of lindane, since it has been reported that the proportion of body weight represented by fat is double that of nonyellow siblings in the closely related lethal yellow *A^y/a* mice (Wolff, 1963). After 30 wk of age, the enhanced storage capacity of the yellow mice resulted in tissue radioactivity concentrations that were significantly lower than those of their nonyellow littermates (Table 1). The significant age × phenotype interaction in liver, blood, and adipose tissue radioactivity appeared to be due to lower concentrations of labeled products in these tissues, which accompanied increasing age in the obese yellow mice when compared to the black and pseudoagouti phenotypes. Reduced blood levels of radioactivity in the yellow mice reflect age-related increases in organ weight, body weight, and fat depots as well as a failure of the rate of lipogenesis to decrease to adult levels as it does in the nonyellow littermates (Yen et al., 1976). Elevated levels of radioactivity in both kidney and liver accompanied aging in all (YS × VY) F_1 hybrid mice. However, the yellow mice had significantly reduced levels when compared to the black and pseudoagouti mice.

At any given age, the level of radioactivity is dependent on the dynamic equilibrium between the rates of metabolism, excretion, and the size of tissue reserves available for storage. For example, hepatic radioactivity was lowest in the 17- and 30-wk-old mice, the ages at which the

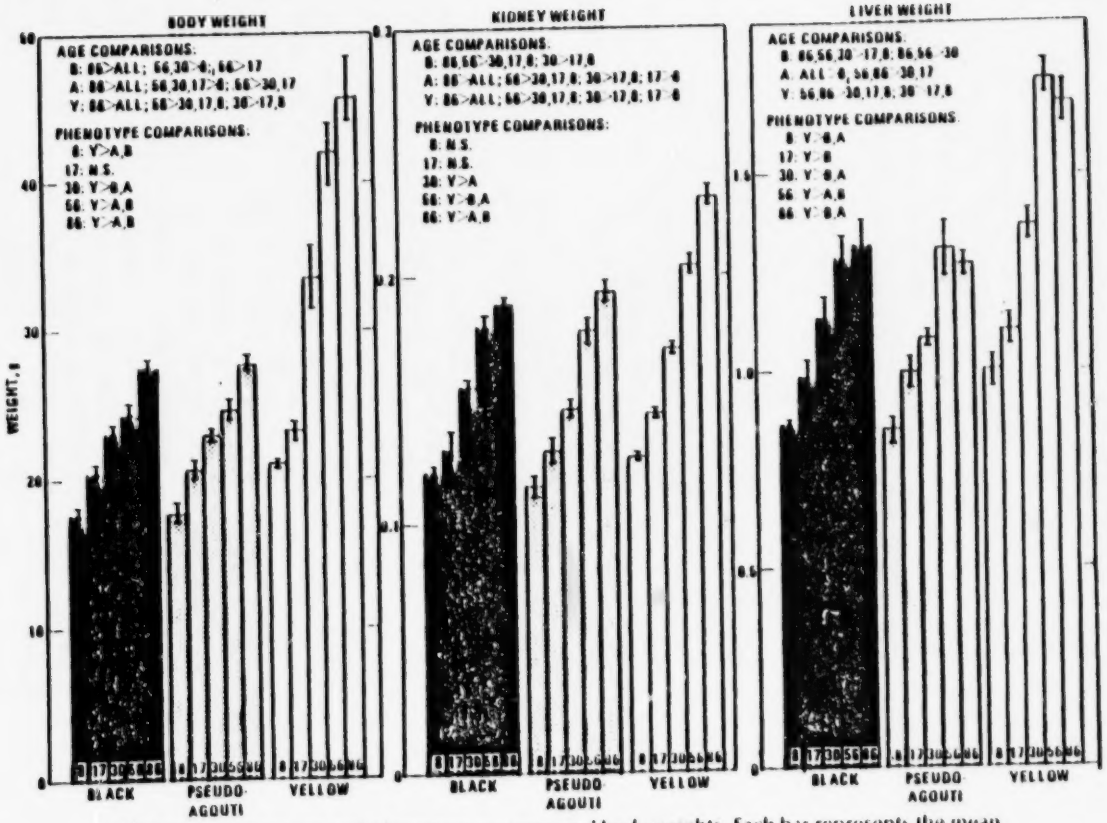

FIGURE 1. Effect of age and phenotype on organ and body weights. Each bar represents the mean weight in grams of the mice from each of 6 cages/phenotype except at 8 and 30 wk of age. In the 8-wk group there were 5 cages of pseudoagouti and 5 cages of yellow mice. At 30 wk of age there were 5 cages of yellow mice. The mean weights of the black *a/a*, pseudoagouti *A^vy/a*, and yellow *A^vy/a* phenotypes are represented by solid, shaded, and open bars, respectively. The vertical lines indicate the standard errors.

maximum percent increase in body weight occurred. Also during this period, the excretion rate remained at peak levels (Table 2). Thus [^{14}C]lindane was rapidly processed and removed from the liver while at the same time being extracted from the blood into enlarged tissue storage depots. Renal radioactivity, on the other hand, began to accumulate at significantly higher levels in the 56-wk-old mice, and this may signal an age-related malfunction of the kidney since urinary radioactivity declined markedly at the same age. Finally, lower levels of radioactivity in the blood of 17-wk-old mice compared to older animals could be due to increased plasma binding of the [^{14}C]lindane in older mice. Many toxic chemicals are bound to plasma proteins, particularly serum albumin (Kasik, 1971), and the albumin synthesis of old animals is 50% higher than that of young ones (Ove et al., 1972; Chen et al., 1973). Lower levels of radioactivity in the blood of 17-wk-old mice compared to that of 8-wk-old mice is probably due to elevated storage in enlarged tissue reserves.

Excretion of lindane-derived radioactivity was significantly influenced by age and phenotype. Table 2 presents the effect of aging and phenotypic influence on the distribution of radioactivity between urine, feces, and expired air. The pseudoagouti and black mice excreted significantly more radioactivity in urine than the yellow mice at every age except that of 17 wk. Significantly higher levels of radioactivity were excreted in feces and expired by the black and pseudoagouti mice when compared to the yellow mice. Urinary radioactivity declined with age in all three phenotypes, while there was a slight elevation in expired air and fecal levels. Calculating the proportion of radioactivity in urine, feces, and expired air from the mean for all phenotypes at each age, it was determined that at 8 wk of age, the urinary radioactivity accounted for about 92% of the total excreted radioactivity while feces and expired air contained 8% and 0.13% of the total, respectively. In the 86-wk-old mice, the proportion of the radioactivity in expired air had doubled, that of feces had risen to 12%, and that excreted in urine had declined to about 87% of the total.

The effect of age on the distribution of radioactivity to five urinary metabolite fractions is summarized in Table 3. With increasing age the proportion of conjugated and polar metabolites increased significantly in the urine while that of the unconjugated metabolites declined. An elevated proportion of conjugated neutral metabolites in the urine of the yellow mice constituted the only significant phenotypic difference in the distribution of radioactivity to these metabolite fractions.

Tables 4 and 5 present the mean excretion of the identified lindane metabolites over all age groups for the three phenotypes. The excretion profiles for all three phenotypes were similar in that the total urinary chlorophenol metabolites (Table 4) exceeded the total urinary alcohol metabolites (Table 5). More than three times as much 2,4,6-trich-

TABLE 1. Effect of Age and Phenotype on Tissue Radioactivity[a]

Tissue	Phe[c]	Age (wk) 8[e]	17[f]	30[g]	56[f]
Liver[a]	B	0.611 ± 0.033	0.451 ± 0.076	0.349 ± 0.034	0.825 ± 0.116
	A	0.549 ± 0.038	0.440 ± 0.032	0.365 ± 0.055	1.22 ± 0.164
	Y	0.438 ± 0.033	0.407 ± 0.041	0.228 ± 0.028	0.420 ± 0.064
Kidney[a]	B	0.046 ± 0.003	0.050 ± 0.007	0.044 ± 0.008	0.078 ± 0.013
	A	0.039 ± 0.003	0.050 ± 0.005	0.048 ± 0.004	0.102 ± 0.014
	Y	0.033 ± 0.002	0.048 ± 0.003	0.033 ± 0.004	0.050 ± 0.007
	$\overline{W}^d$	0.040 ± 0.002	0.050 ± 0.003	0.042 ± 0.003	0.077 ± 0.008
Blood[b]	B	0.121 ± 0.011	0.082 ± 0.009	0.089 ± 0.010	0.135 ± 0.017
	A	0.123 ± 0.013	0.069 ± 0.014	0.118 ± 0.013	0.213 ± 0.032
	Y	0.105 ± 0.017	0.084 ± 0.010	0.065 ± 0.008	0.077 ± 0.017
Adipose[b]	B	5.02 ± 0.09	3.68 ± 0.22	3.94 ± 0.20	4.15 ± 0.70
	A	4.93 ± 0.30	3.52 ± 0.20	4.38 ± 0.43	4.65 ± 0.48
	Y	2.93 ± 0.12	3.12 ± 0.15	1.68 ± 0.23	1.12 ± 0.15

[a] Values are % administered dose/organ ± SE.
[b] Values are % administered dose/g tissue ± SE.
[c] Phenotypes: B, black a/a mice; A, pseudoagouti A^{vy}/a mice; Y, yellow A^{vy}/a mice.
[d] Mean age value for all phenotypes ± SE.
[e] Data are derived from pooled tissue samples from each of six cages of black, five cages of pseudoagouti, and five cages of yellow mice.
[f] Data are derived from pooled tissue samples from each of six cages of black, six cages of pseudoagouti, and six cages of yellow mice.
[g] Data are derived from pooled tissue samples from each of six cages of black, six cages of pseudoagouti, and five cages of yellow mice.

lorophenol (2,4,6-TCP) was excreted as the next most concentrated chlorophenol, 2,3,5-trichlorophenol (2,3,5-TCP), for all three phenotypes. Similarly, from 2.8 to 40 times as much 2,3,4,6-tetrachloro-2-cyclohexen-1-ol (2,3,4,6-TCCOL) as the sum of the other alcohol metabolites was excreted by the three phenotypes at the different ages. With the exception of 2,3,4,5-tetrachlorophenol (2,3,4,5-TTCP), the yellow mice excreted significantly less of the other chlorophenol metabolites than did the black and pseudoagouti mice. Furthermore, conjugation of the lindane metabolites as glucuronides and sulfates was signifi-

TABLE 1. Effect of Age and Phenotype on Tissue Radioactivity[a] (*Continued*)

Age (wk)		Treatment comparison[c]	
86[b]	$\bar{P}$[b]	Age[c,d]	Phenotype[c,d]
1.016 ± 0.174		B: 86,56 > 17,30;	8: N.S.
		86 > 8; 8 > 17,30	17: N.S.
0.782 ± 0.061		A: 56 > All;	30: A,B > Y
		86 > 17,30; 8 > 30	56: A,B > Y; A > B
0.481 ± 0.022		Y: A11 > 30	86: B,A > Y
		(Age × phenotype interaction)	
0.100 ± 0.022	0.064 ± 0.007	B: 86,56 > 17,8,30	8: N.S.
		A: 56,86 > 17,30,8	17: N.S.
0.091 ± 0.003	0.067 ± 0.006	Y: 86 > All;	30: N.S.
		56,17 > 30	56: A,B > Y
0.085 ± 0.007	0.051 ± 0.004		86: N.S.
0.092 ± 0.007		$\bar{W}$: 86 > All;	$\bar{P}$: A,B > Y
		56 > 17,30,8	
0.110 ± 0.007		B: 56,30 > 17	8: N.S.
		56 > 30	17: N.S.
0.113 ± 0.007		A: 56 > All	30: A > Y
		8,30,86 > 17	56: A,B > Y; A > B
0.053 ± 0.005		Y: 8 > 86	86: A,B > Y
		(Age × phenotype interaction)	
3.76 ± 0.28		B: 8 > All	8: B,A > Y
		A: 8,56,30 > 17,86	17: N.S.
3.29 ± 0.22		Y: 17,8 > 30,86,56	30: A,B > Y
			56: A,B > Y
1.34 ± 0.10			86: B,A > Y
		(Age × phenotype reaction)	

[a] Mean phenotype value for all ages ± SE.

[c] Comparisons are significantly different at $p < 0.05$ by least square means in the general linear models procedure. When there were no age × phenotype interactions, $\bar{W}$ and $\bar{P}$ comparisons were tested at $p < 0.05$ by Duncan's multiple-range test.

[d] N.S. indicates the comparisons were not significantly different.

cantly more important in the yellow mice than in their black and pseudoagouti littermates. The mean proportion of the chlorophenol and alcohol metabolites that were conjugated was 49.3 ± 3.9% for the yellow mice, compared to 40.7 ± 4.2% and 35.0 ± 3.5% for the pseudoagouti and black mice, respectively.

While excretion of the chlorophenols declined significantly with increasing age (Table 4), excretion of the major alcohol metabolite, 2,3,4,6-TCCOL, was significantly increased (Table 5).

Table 6 presents the effect of age and phenotype on two key enzymatic pathways in the hepatic metabolism of lindane. Dehydrogenation is a rate-limiting step in the metabolism of this insecticide to 2,3,4,6-

TABLE 2. Effect of Age and Phenotype on Excretion of Radioactivity[a]

Excreta	Phe[b]	Age (wk) 8[d]	17[e]	30[f]
Urine	B	53.3 ± 3.6	48.1 ± 2.5	48.2 ± 2.9
	A	51.3 ± 2.1	44.8 ± 2.9	51.1 ± 3.5
	Y	42.8 ± 1.4	42.3 ± 1.9	31.1 ± 2.4
	$\overline{W}$[c]	49.4 ± 1.9	45.0 ± 1.4	44.2 ± 2.7
Feces	B	4.83 ± 0.50	3.07 ± 0.26	5.47 ± 0.60
	A	4.95 ± 0.22	3.48 ± 0.56	4.69 ± 0.70
	Y	3.57 ± 0.60	3.70 ± 0.42	2.63 ± 0.60
	$\overline{W}$[c]	4.47 ± 0.30	3.42 ± 0.24	4.36 ± 0.45
Expired air	B	0.081 ± 0.007	0.099 ± 0.016	0.119 ± 0.011
	A	0.075 ± 0.019	0.084 ± 0.014	0.088 ± 0.017
	Y	0.045 ± 0.002	0.067 ± 0.009	0.046 ± 0.006
	$\overline{W}$[c]	0.068 ± 0.007	0.083 ± 0.008	0.086 ± 0.010

[a] Values are mean % of administered dose excreted/24 h ± SE.

[b] Phenotypes: B, black a/a mice; A, pseudoagouti A^{vy}/a mice; Y, yellow A^{vy}/a mice.

[c] Mean age value for all phenotypes ± SE.

[d] Data are derived from pooled samples from each of six cages of black, five cages of pseudoagouti, and five cages of yellow mice.

[e] Data are derived from pooled samples from each of six cages of black, six cages of pseudoagouti, and six cages of yellow mice.

[f] Data are derived from pooled samples from each of six cages of black, six cages of pseudoagouti, and five cages of yellow mice.

and 2,3,4,5-TTCP and 2,4-, 2,5-, 3,4-, 2,3,5-, and 2,4,5-chlorophenyl mercapturic acids and PCCOL (Chadwick et al., 1975b, 1981). Dechlorination plays a role in the metabolism of lindane to mono- and trichlorophenyl mercapturic acids (Kurihara et al., 1979). When the activity was expressed per gram of liver, only the dechlorination of lindane showed significant changes. The significant age × phenotype interaction associated with the in vitro dechlorination of lindane was due to the greater age-related increase in the dechlorination of lindane by the yellow mice. Only liver preparations from the obese yellow mice had significantly higher activity for the dechlorination of lindane at 86 wk of age than at 8 wk of age. This increase was over four times greater than that

TABLE 2. Effect of Age and Phenotype on Excretion of Radioactivity[a] (*Continued*)

Age (wk)			Treatment comparison[b]	
56[e]	86[e]	$\bar{P}$[e]	Age[b,f]	Phenotype[b,f]
34.4 ± 3.3	41.4 ± 2.5	45.1 ± 1.7	B: 8,30,17 > 56; 8 > 86	8: B,A > Y 17: N.S.
33.9 ± 3.5	39.3 ± 2.5	43.8 ± 1.8	A: 8,30 > 86,56; 17 > 56	30: A,B > Y 56: B,A > Y
22.5 ± 1.1	31.4 ± 2.3	33.8 ± 1.7	Y: All > 56; 8,17 > 86,30,56	86: B,A > Y
30.3 ± 2.0	37.4 ± 1.7		$\bar{W}$: 8,17,30 > 86,56 8 > 30; 86 > 56	$\bar{P}$: B,A > Y
6.49 ± 1.20	5.43 ± 1.06	5.06 ± 0.39	B: 56 > 17	8: N.S.
			A: 86,56 > 17	17: N.S.
6.13 ± 1.25	6.49 ± 1.23	5.15 ± 0.43	Y: N.S.	30: B > Y
				56: N.S.
5.19 ± 2.07	3.20 ± 0.50	3.70 ± 0.48		86: A > Y
5.94 ± 0.86	5.04 ± 0.63		$\bar{W}$: 56 > 17	$\bar{P}$: A,B > Y
0.074 ± 0.008	0.145 ± 0.012	0.103 ± 0.007	B: 86,30 > 8,56; 86 > 17	8: N.S. 17: N.S.
0.080 ± 0.013	0.114 ± 0.021	0.089 ± 0.007	A: 86 > 8	30: B,A > Y
			Y: 86 > 56	56: A > Y
0.040 ± 0.007	0.081 ± 0.015	0.057 ± 0.005		86: B > Y
0.065 ± 0.007	0.113 ± 0.011		$\bar{W}$: 86 > All	$\bar{P}$: B,A > Y

[a] Mean phenotype value for all ages ± SE.

[b] Comparisons are significantly different at $p < 0.05$ by least square means in the general linear models procedure. When there were no age × phenotype interactions, $\bar{W}$ and $\bar{P}$ comparisons were tested at $p < 0.05$ by Duncan's multiple-range test.

[f] N.S. indicates the comparisons were not significantly different.

[f] The meaning of this test should be viewed with caution, since transformation did not solve the heterogeneity of variance problem in data from the yellow mice.

observed for the black and pseudoagouti mice. When enzyme activity was expressed on the basis of the whole liver, both the dehydrogenation and dechlorination of lindane were significantly elevated in the yellow mice. The effect of aging on these enzymes was not clearcut, however. After an initial increase at 17 wk, activity of both enzymes declined until 86 wk when there was a marked recovery.

Aging resulted in significant reduction in hepatic phospholipids for the three ages at which they were analyzed (Table 7). The oldest mice had 19% less total hepatic phospholipids, 22% less sphingomyelin (SP), 16% less phosphatidylcholine (PC), 30% less combined phosphatidylserine/phosphatidylinositol (PS/PI), and 18% less phosphatidylethanolamine (PE). There were no significant differences among the phenotypes in these reductions.

TABLE 3. Effect of Age and Phenotype on Distribution of Radioactivity in Urine (% of Urinary ^{14}C ± SE)

Urine extract	Phe[a]	Age (wk) 8[c]	17[d]	30[e]
Free neutrals (Radioactivity extracted from acid urine (pH = 2) that does not partition into 0.15 *N* NaOH.)	B	2.46 ± 0.30	3.82 ± 0.65	2.86 ± 0.27
	A	2.42 ± 0.35	3.35 ± 0.33	3.59 ± 0.23
	Y	2.25 ± 0.44	2.49 ± 0.30	3.72 ± 0.39
	$\overline{W}$[b]	2.38 ± 0.20	3.20 ± 0.28	3.37 ± 0.18
Conjugated neutrals (Radioactivity extracted from hydrolyzed urine that does not partition into 0.15 *N* NaOH.)	B	2.71 ± 0.25	1.75 ± 0.23	1.32 ± 0.25
	A	2.79 ± 0.27	0.89 ± 0.11	1.26 ± 0.51
	Y	3.27 ± 0.26	2.22 ± 0.45	1.54 ± 0.57
	$\overline{W}$[b]	2.91 ± 0.15	1.62 ± 0.21	1.36 ± 0.25
FCP (Radioactivity extracted from acid urine (pH = 2) that partitions into 0.15 *N* NaOH; FCP, free chlorophenol.)	B	29.2 ± 2.2	37.8 ± 3.4	41.0 ± 2.5
	A	28.3 ± 2.8	38.9 ± 3.7	44.6 ± 2.4
	Y	25.5 ± 2.7	32.7 ± 1.9	44.8 ± 2.1
	$\overline{W}$[b]	27.8 ± 1.4	36.5 ± 1.8	43.4 ± 1.4
CCP (Radioactivity extracted from hydrolyzed urine which partitions into 0.15 *N* NaOH; CCP, conjugated chlorophenol.)	B	18.9 ± 2.2	15.9 ± 3.3	14.1 ± 2.5
	A	21.4 ± 2.6	15.6 ± 3.4	10.9 ± 1.7
	Y	24.1 ± 2.5	20.2 ± 2.3	10.3 ± 1.1
	$\overline{W}$[b]	21.3 ± 1.4	17.2 ± 1.7	11.8 ± 1.1
Polar metabolites (Radioactivity in residual hydrolyzed urine after extraction.)	B	46.8 ± 0.2	40.7 ± 0.2	40.8 ± 0.6
	A	45.0 ± 0.5	41.3 ± 0.9	39.6 ± 0.8
	Y	44.8 ± 1.0	42.4 ± 1.2	39.6 ± 2.4
	$\overline{W}$[b]	45.6 ± 0.4	41.5 ± 0.6	40.0 ± 0.7

[a] Phenotypes: B, black a/a mice; A, pseudoagouti A^{vy}/a mice; Y, yellow A^{vy}/a mice.

[b] Mean age value for all phenotypes ± SE.

[c] Data are derived from pooled urine samples from each of six cages of black, five cages of pseudoagouti, and five cages of yellow mice.

[d] Data are derived from pooled urine samples from each of six cages of black, six cages of pseudoagouti, and six cages of yellow mice.

[e] Data are derived from pooled urine samples from each of six cages of black, six cages of pseudoagouti, and five cages of yellow mice.

TABLE 3. Effect of Age and Phenotype on Distribution of Radioactivity in Urine[a] (*Continued*)

Age (wk)			Treatment comparison[g]	
56[d]	86[d]	$\overline{P}$[f]	Age[g,h]	Phenotype[g,h]
3.59 ± 0.91	6.52 ± 3.20	3.85 ± 0.69	B: 86 > 8	8: N.S. 17: N.S.
2.80 ± 0.63	3.36 ± 0.59	3.13 ± 0.21	A: N.S.	30: N.S. 56: N.S.
4.35 ± 0.83	1.97 ± 0.33	2.95 ± 0.28	Y: 56,30 > 86; 56 > 8	86: B,A > Y
3.58 ± 0.46	3.95 ± 1.12		$\overline{W}$: N.S.	$\overline{P}$: N.S.
3.93 ± 0.17	4.58 ± 0.96	2.86 ± 0.30	B: 86,56 > 8,17,30; 8 > 30	8: N.S. 17: Y,B > A
3.80 ± 0.39	5.29 ± 0.43	2.81 ± 0.35	A: 86 > All; 56,8 > 30,17	30: N.S. 56: N.S.
4.57 ± 0.18	5.43 ± 0.29	3.48 ± 0.32	Y: 86,56,8 > 30; 86,56 > 17; 86 > 8	86: N.S.
4.10 ± 0.17	5.10 ± 0.35		$\overline{W}$: 86 > All; 56 > 8,17,30; 8 > 17,30	$\overline{P}$: Y > A,B
29.0 ± 2.5	25.6 ± 2.4	32.5 ± 1.5	B: 30,17 > 8,56,86	8: N.S. 17: N.S.
21.5 ± 3.2	22.0 ± 2.0	31.2 ± 2.1	A: 30,17 > 8,86,56 Y: 30 > All;	30: N.S. 56: B > A
26.9 ± 1.5	18.7 ± 1.9	29.3 ± 1.8	17,56 > 86	86: N.S.
25.8 ± 1.5	22.1 ± 1.3		$\overline{W}$: 30 > All; 17 > 8,56,86; 8 > 86	$\overline{P}$: N.S.
20.8 ± 2.5	18.7 ± 2.3	17.7 ± 1.2	B: N.S. A: 56,86 > 17,30; 8 > 30	8: N.S. 17: N.S. 30: N.S.
25.8 ± 2.6	23.8 ± 2.3	19.5 ± 1.5	Y: All > 30	56: N.S. 86: N.S.
21.0 ± 2.1	23.8 ± 2.3	20.1 ± 1.3		
22.6 ± 1.4	22.1 ± 1.4		$\overline{W}$: 56,86,8 > 17,30; 17 > 30	$\overline{P}$: N.S.
42.7 ± 1.3	44.6 ± 2.6	43.1 ± 0.7	B: 8,86 > 30,17; 8 > 56	8: N.S. 17: N.S.
46.1 ± 2.2	45.6 ± 0.8	43.5 ± 0.7	A: 56,86 > 17,30; 8 > 30	30: N.S. 56: N.S.
43.2 ± 0.6	50.1 ± 0.9	44.1 ± 0.9	Y: 86 > All; 8 > 30	86: Y > A,B
44.0 ± 0.9	46.7 ± 1.1		$\overline{W}$: 86 > 56,17,30; 8,56 > 17,30	$\overline{P}$: N.S.

[f] Mean phenotype value for all ages ± SE.

[g] Comparisons are significantly different at $p < 0.05$ by least square means in the general linear models procedures. When there were no age × phenotype interactions, the $\overline{W}$ and $\overline{P}$ comparisons were tested at $p < 0.05$ by Duncan's multiple range test.

[h] N.S. indicates the comparisons were not significantly different.

[i] The meaning of this test should be viewed with caution, since transformation did not solve the heterogeneity of variance problem in data from the black mice.

TABLE 4. Effect of Age and Phenotype on the Excretion of Chlorophenol Metabolites (% Adm. Dose ± SE)

Metabolite	Phe[a]	Age (wk) 8[c]	17[d]	30[e]
2,4,6-trichloro-phenol (2,4,6-TCP)	B	10.27 ± 0.64	6.43 ± 0.99	11.16 ± 0.90
	A	9.94 ± 0.46	6.91 ± 1.25	10.19 ± 1.84
	Y	7.84 ± 0.42	6.04 ± 0.46	6.23 ± 0.33
	$\overline{W}$[b]	9.41 ± 0.41	6.46 ± 0.53	9.37 ± 0.86
2,3,5-trichloro-phenol (2,3,5-TCP)	B	2.56 ± 0.17	1.70 ± 0.27	2.27 ± 0.24
	A	2.50 ± 0.21	1.84 ± 0.28	2.31 ± 0.17
	Y	2.02 ± 0.12	1.60 ± 0.15	1.30 ± 0.08
	$\overline{W}$[b]	2.37 ± 0.11	1.71 ± 0.13	2.00 ± 0.15
2,4,5-trichloro-phenol (2,4,5-TCP)	B	2.09 ± 0.15	1.35 ± 0.21	1.97 ± 0.22
	A	2.09 ± 0.18	1.56 ± 0.20	1.97 ± 0.15
	Y	1.62 ± 0.08	1.53 ± 0.13	1.26 ± 0.11
	$\overline{W}$[b]	1.94 ± 0.10	1.48 ± 0.10	1.76 ± 0.12
2,3,4,6-tetrachloro-phenol (2,3,4,6-TTCP)	B	1.04 ± 0.07	0.69 ± 0.09	0.68 ± 0.09
	A	0.98 ± 0.09	0.77 ± 0.11	0.70 ± 0.07
	Y	0.80 ± 0.04	0.79 ± 0.05	0.47 ± 0.06
	$\overline{W}$[b]	0.95 ± 0.05	0.75 ± 0.05	0.62 ± 0.05
2,3,4,5-tetrachloro-phenol (2,3,4,5-TTCP)	B	0.172 ± 0.011	0.133 ± 0.030	0.036 ± 0.028
	A	0.336 ± 0.193	0.174 ± 0.037	0.010 ± 0.005
	Y	0.136 ± 0.006	0.145 ± 0.016	0.013 ± 0.006
	$\overline{W}$[b]	0.212 ± 0.060	0.151 ± 0.016	0.020 ± 0.010

[a] Phenotypes: B, black *a/a* mice; A, pseudoagouti A^{vy}/a mice; Y, yellow A^{vy}/a mice.

[b] Mean age value for all phenotypes ± SE.

[c] Data are derived from pooled urine samples from each of six cages of black, five cages of pseudoagouti, and five cages of yellow mice.

[d] Data are derived from pooled urine samples from each of six cages of black, six cages of pseudoagouti, and six cages of yellow mice.

[e] Data are derived from pooled urine samples from each of six cages of black, six cages of pseudoagouti, and five cages of yellow mice.

[f] Mean phenotype values for all ages ± SE.

[g] Comparisons are significantly different at $p < 0.05$ by least square means in the general linear models procedure. When there were no age × phenotype interactions, $\overline{W}$ and $\overline{P}$ comparisons were tested at $p < 0.05$ by Duncan's multiple-range test.

[h] N.S. indicates the comparisons were not significantly different.

TABLE 4. Effect of Age and Phenotype on the Excretion of Chlorophenol Metabolites (*Continued*)

Age (wk)			Treatment comparison[a]	
56[a]	86[a]	$\bar{P}'$	Age[a,b]	Phenotype[a,b]
11.34 ± 1.20	3.67 ± 0.33	8.57 ± 0.67	B: All > 86; 56,30,8 > 17	8: N.S. 17: N.S.
12.78 ± 2.47	3.67 ± 0.30	8.66 ± 0.88	A: All > 86; 56 > 17	30: B > Y 56: N.S.
8.50 ± 1.20	2.83 ± 0.31	6.24 ± 0.48	Y: All > 86	86: N.S.
10.9 ± 1.03	3.39 ± 0.20		$\bar{W}$: All > 86; 56,8,30 > 17	$\bar{P}$: A,B > Y
2.79 ± 0.43	0.83 ± 0.09	2.03 ± 0.17	B: All > 86; 56,8 > 17	8: N.S. 17: N.S.
3.15 ± 0.72	1.01 ± 0.10	2.15 ± 0.21	A: All > 86; 56 > 17	30: A,B > Y 56: N.S.
1.87 ± 0.38	0.70 ± 0.08	1.48 ± 0.13	Y: All > 86; 8 > 30	86: N.S.
2.60 ± 0.32	0.84 ± 0.06		$\bar{W}$: All > 86; 56,8 > 17	$\bar{P}$: A,B > Y
1.92 ± 0.23	0.99 ± 0.10	1.66 ± 0.11	B: 8,30,56 > 86	8: N.S. 17: N.S.
2.06 ± 0.47	1.40 ± 0.40	1.81 ± 0.13	A: 8,56 > 86; 8,30 > 17	30: A,B > Y 56: A > Y
1.34 ± 0.18	0.83 ± 0.10	1.31 ± 0.08	Y: 8,17 > 86	86: N.S.
1.77 ± 0.19	1.07 ± 0.10		$\bar{W}$: All > 86; 8 > 17	$\bar{P}$: A,B > Y
0.61 ± 0.05	0.38 ± 0.05	0.68 ± 0.05	B: All > 86; 8 > All	8: B > Y 17: N.S.
0.71 ± 0.14	0.41 ± 0.04	0.71 ± 0.05	A: All > 86; 8 > 56,30	30: A > Y 56: A,B > Y
0.36 ± 0.07	0.27 ± 0.03	0.53 ± 0.05	Y: 8,17 > 30,56,86	86: N.S.
0.56 ± 0.06	0.35 ± 0.03		$\bar{W}$: All > 86; 17,8 > 56,30	$\bar{P}$: A,B > Y
0.046 ± 0.023	0.054 ± 0.037	0.088 ± 0.015	B: 8,17 > 86,56,30 A: 8,17 > 56,30,86	8: N.S. 17: N.S.
0.036 ± 0.014	0.009 ± 0.001	0.105 ± 0.039		30: N.S. 56: N.S.
0.021 ± 0.011	0.005 ± 0.001	0.063 ± 0.013	Y: 17,8 > 56,30,86	86: N.S.
0.034 ± 0.009	0.023 ± 0.013		$\bar{W}$: 8 > 56,86,30; 17 > 56,86,30	$\bar{P}$: N.S.

TABLE 5. Effect of Age and Phenotype on the Excretion of Alcohol Metabolites and Unaltered Lindane[a]

Metabolite[b]	Phe[c]	Age (wk)		
		8[e]	17[f]	30[f]
2,3,4,6-TCCOL	B	1.02 ± 0.21	1.25 ± 0.17	0.52 ± 0.17
	A	1.14 ± 0.14	0.38 ± 0.14	1.77 ± 1.69
	Y	0.96 ± 0.12	0.95 ± 0.20	0.42 ± 0.39
	$\overline{W}^d$	1.04 ± 0.09	0.86 ± 0.13	0.93 ± 0.59
2,4,5,6-TCCOL	B	0.174 ± 0.015	0.173 ± 0.026	0.011 ± 0.003
	A	0.165 ± 0.023	0.107 ± 0.024	0.021 ± 0.017
	Y	0.103 ± 0.017	0.125 ± 0.017	0.010 ± 0.006
PCCOL	B	0.054 ± 0.013	0.073 ± 0.010	0.177 ± 0.137
	A	0.064 ± 0.008	0.016 ± 0.007	0.126 ± 0.106
	Y	0.069 ± 0.023	0.075 ± 0.030	0.033 ± 0.021
	$\overline{W}^d$	0.062 ± 0.009	0.055 ± 0.012	0.117 ± 0.060
γ-HCH	B	0.033 ± 0.010	0.027 ± 0.003	0.028 ± 0.008
	A	0.033 ± 0.015	0.018 ± 0.004	0.024 ± 0.009
	Y	0.015 ± 0.004	0.027 ± 0.006	0.009 ± 0.002

[a] Values are % of administered dose ± SE.

[b] Metabolite abbreviations are: 2,3,4,6-TCCOL, 2,3,4,6-tetrachloro-2-cyclohexen-1-ol; 2,4,5,6-TCCOL, 2,4,5,6-tetrachloro-2-cyclohexen-1-ol; PCCOL, 2,3,4,5,6-pentachloro-2-cyclohexen-1-ol; γ-HCH, γ-hexachlorocyclohexane (lindane).

[c] Phenotypes: B, black *a/a* mice; A, pseudoagouti A^w/a mice; Y, yellow A^w/a mice.

[d] Mean age value for all phenotypes ± SE.

[e] Data are derived from pooled urine samples from each of six cages of black, five cages of pseudoagouti, and five cages of yellow mice.

[f] Data are derived from pooled urine samples from each of six cages of black, six cages of pseudoagouti, and six cages of yellow mice.

TABLE 5. Effect of Age and Phenotype on the Excretion of Alcohol Metabolites and Unaltered Lindane[a] (*Continued*)

Age (wk)			Treatment comparison[c]	
56[c]	86[c]	$\bar{P}$[b]	Age[c,d]	Phenotype[c,d]
1.92 ± 0.44	2.46 ± 0.69	1.43 ± 0.21	B: 86,56 > 30	8: N.S. 17: B > A
1.96 ± 0.48	3.38 ± 0.62	1.74 ± 0.41	A: 86,56 > 17; 86 > 30,8	30: N.S. 56: N.S.
1.61 ± 0.19	2.66 ± 0.40	1.36 ± 0.19	Y: 86 > 8,17,30; 56 > 30	86: N.S.
1.83 ± 0.22	2.83 ± 0.33		$\overline{W}$: 86 > 8,30,17; 56 > 30,17	$\bar{P}$: N.S.
0.010 ± 0.003	0.008 ± 0.002		B: 8,17 > 30,56,86	8: B,A > Y 17: B > A
0.014 ± 0.005	0.011 ± 0.003		A: 8 > All; 17 > 30,56,86	30: N.S. 56: N.S.
0.011 ± 0.002	0.011 ± 0.002		Y: 17,8 > 86,56,30 (Age × phenotype interaction)	86: N.S.
0.180 ± 0.071	0.092 ± 0.027	0.115 ± 0.031	B: N.S.	8: N.S. 17: Y,B > A
0.096 ± 0.019	0.071 ± 0.027	0.075 ± 0.023	A: 56,86,8 > 17	30: N.S. 56: N.S.
0.075 ± 0.007	0.093 ± 0.012	0.070 ± 0.009	Y: 86,56 > 30	86: N.S.
0.177 ± 0.025	0.085 ± 0.013		$\overline{W}$: 56,86 > 30; 56 > 17	$\bar{P}$: N.S.
0.087 ± 0.037	0.024 ± 0.006		B: N.S.	8: N.S. 17: N.S.
0.073 ± 0.028	0.013 ± 0.002		A: 56 > 30,17,86	30: B,A > Y 56: N.S.
0.300 ± 0.208	0.007 ± 0.001		Y: 56,17,8 > 30,86; 56 > 8 (Age × phenotype interaction)	86: B,A > Y

[a] Data are derived from pooled urine samples from each of six cages of black, six cages of pseudoagouti, and five cages of yellow mice.

[b] Mean phenotype value for all ages ± SE.

[c] Comparisons are significantly different at $p < 0.05$ by least square means in the general linear models procedure. When there were no age × phenotype interactions, $\overline{W}$ and $\bar{P}$ comparisons were tested at $p < 0.05$ by Duncan's multiple-range test.

[d] N.S. indicates the comparisons were not significantly different.

DISCUSSION

Results of this study indicate that the differences in lindane metabolism and disposition observed in the (YS × VY) F_1 hybrid mice were associated with obesity and were not due to genotypic effects. Effects associated with the A^{vy}/a genotype were examined by comparing the pseudoagouti A^{vy}/a with the black a/a mice. These mice are both lean and resemble each other in many of the physiological and biochemical parameters that have been examined (Roberts et al., 1984; Wolff, 1971; Wolff and Suber, 1983). Since no significant differences in the metabolism and disposition of lindane were observed between the black and pseudoagouti mice, no role could be attributed to the direct effects of the genotype. On the other hand, it appeared that obesity did play a significant role in the metabolism and disposition of lindane. Comparison of the genetically identical obese yellow and lean pseudoagouti sibs indicate that obesity, a nonobligatory phenotypic effect of the A^{vy}/a genotype, was associated with significant alterations in tissue storage (Table 1) and excretion (Table 2) of lindane and its metabolites.

It has been demonstrated that the viable yellow A^{vy} mutation enhances a predisposition to the formation of hepatomas in the mouse (Heston and Vlahakis, 1968; Wolff and Pitot, 1972). This effect was thought to be related to an alteration in normal growth. It has been reported that the percent of carcass fat in the closely related lethal yellow A^{y}/a female mouse is over twice as great as that in the nonyellow a/a sister (Wolff, 1963). The characteristic obesity of the yellow mouse seems to be due to failure of the rate of lipogenesis to decrease to adult levels as it does in the nonyellow littermates (Yen et al., 1976). Thus the capacity of the yellow mouse to store lipid-soluble carcinogens, procarcinogens, and/or their metabolites is greatly enhanced and could be linked to its greater susceptibility to lindane-induced hepatomas (Wolff, 1985). A significantly higher body burden would be particularly important in older animals where both xenobiotic metabolism (Kato and Takanaka, 1968a,b; Chadwick et al., 1975a; Schmucker and Wang, 1980; Rikans and Notley, 1982) and susceptibility to hepatotoxic injury (Rikans, 1984) are altered.

In this study the overall excretion of lindane metabolites declined with increasing age (Tables 2, 4, 5), while tissue storage increased (Table 1). Conjugation and excretion of lindane metabolites as glucuronides, sulfates, and mercapturic acids became more important in the older mice (Table 3) and may signal increased reabsorption of unconjugated metabolites from the kidney. Due to the ease with which conjugation reactions can be saturated (Williams, 1978), increased reliance on conjugation mechanisms to eliminate the metabolites of lindane may enhance the potential of unconjugated active metabolites to in-

duce hepatic lesions. It is notable that the proportion of conjugated metabolites in the urine of the 86-wk-old yellow mice was significantly higher than in that from the black or pseudoagouti mice (Table 3). In addition, the urinary level of the chlorophenol metabolites was significantly reduced in the oldest mice (Table 4), while that of the more lipid-soluble 2,3,4,6-TCCOL was significantly increased (Table 6). The carcinogenic potential of this metabolite and its intermediate(s) has not been tested. Finally, aging reduced the phospholipid content of the liver, which may alter its ability to process lindane and/or its susceptibility to toxic insult from this chemical (Table 7). Hepatic phospholipids are not only structural and storage components but may also play a role in the regulation of enzyme activity and membrane permeability (Op Den Kamp et al., 1967; Dallner and Ernster, 1968; Papahadjopoulos, 1973; Vaino, 1973).

The yellow mice had significantly higher hepatic enzyme activity than their black and pseudoagouti sisters (Table 6). While the increase in the in vitro dechlorination of lindane by yellow mice at 86 wk of age was significantly greater than that of their nonyellow siblings, the in vitro dehydrogenation of lindane was greater only when activity was expressed in terms of the whole liver. Dechlorination is believed to play an essential role in the metabolism of lindane to the mono- and trichlorophenyl mercapturic acids (Kurihara et al., 1979). Moreover, Kurihara and his associates (1980) have demonstrated that this enzymatic pathway is responsible for the metabolism of the lindane intermediate metabolites, hexachlorocyclohexene and pentachlorocyclohexene, to 1,2,4-trichlorobenzene and 1,4-dichlorobenzene, respectively. One of these metabolites, 1,2,4-trichlorobenzene, has been reported to be hepatotoxic (Cameron et al., 1937) and to deplete hepatic glutathione in a dose-related manner (Kitchin and Ebron, 1983). Moreover, Kohli et al. (1976) proposed a pathway for the biotransformation of 1,2,4-trichlorobenzene through an epoxide intermediate. Hepatic necrosis by halogenated aromatic hydrocarbons is presumably due to covalent binding of halobenzene epoxides to liver macromolecules (Brodie et al., 1971; Jollow et al., 1974; Oesch et al., 1973; Reid and Krishna, 1973). The epoxide is inactivated by conversion to glutathione conjugates (Jollow et al., 1974; Zampaglione et al., 1973). Therefore, since Wolff and Suber (1983) have reported that the viable yellow A^{vy}/a mouse, employed in this study, has lower hepatic glutathione *S*-transferase activity than its nonyellow littermates, the risk from increased binding of chemically reactive metabolites to liver macromolecules may be elevated in these animals.

In summary, both age and phenotype influenced the metabolism and disposition of lindane in (YS × VY) F_1 hybrid mice. The greater susceptibility of the aging, obese, yellow A^{vy}/a mouse to lindane-induced hepatomas (Wolff, 1985) was accompanied by (1) increased

TABLE 6. Effect of Age and Phenotype on in Vitro Enzyme Activity

Enzyme assay	Phe[e]	Age (wk)		
		8[g]	17[h]	30[i]
Dehyd.[a]	B	1.27 ± 0.08	1.37 ± 0.28	1.06 ± 0.09
	A	1.24 ± 0.05	1.79 ± 0.26	1.12 ± 0.08
	Y	1.26 ± 0.06	1.46 ± 0.23	1.23 ± 0.09
	$\overline{W}$[f]	1.26 ± 0.03	1.54 ± 0.15	1.13 ± 0.051
Dehyd.[b]	B	1.10 ± 0.06	1.37 ± 0.30	1.21 ± 0.13
	A	1.05 ± 0.04	1.76 ± 0.25	1.20 ± 0.08
	Y	1.26 ± 0.06	1.59 ± 0.26	1.66 ± 0.09
	$\overline{W}$[f]	1.14 ± 0.04	1.58 ± 0.15	1.34 ± 0.078
Dechlor.[c]	B	0.184 ± 0.037	0.415 ± 0.052	0.283 ± 0.054
	A	0.113 ± 0.041	0.680 ± 0.078	0.585 ± 0.160
	Y	0.134 ± 0.008	0.769 ± 0.119	0.924 ± 0.218
Dechlor.[d]	B	0.158 ± 0.033	0.411 ± 0.059	0.321 ± 0.065
	A	0.093 ± 0.032	0.675 ± 0.088	0.622 ± 0.167
	Y	0.133 ± 0.007	0.847 ± 0.145	1.23 ± 0.280

[a] In vitro dehydrogenation of lindane to γ-1,2,3,4,5,6-hexachlorocyclohex-1-ene (γ-HCCH). Values are mean μg γ-HCCH/g liver · 30 min, ±SE.

[b] In vitro dehydrogenation of lindane to γ-1,2,3,4,5,6-hexachlorocyclohex-1-ene (γ-HCCH). Values are mean μg γ-HCCH/liver · 30 min, ±SE.

[c] In vitro dechlorination of lindane to γ-3,4,5,6-tetrachlorocyclohex-1-ene (γ-TCCH). Values are mean μg γ-TCCH/g liver · 30 min, ±SE.

[d] In vitro dechlorination of lindane to γ-3,4,5,6-tetrachlorocyclohex-1-ene (γ-TCCH). Values are mean μg γ-TCCH/liver · 30 min, ±SE.

[e] Phenotypes: B, black *a/a* mice; A, pseudoagouti A^{vy}/a mice; Y, yellow A^{vy}/a mice.

[f] Mean age value for all phenotypes ± SE.

[g] Data are derived from the 9000 × g supernatant of pooled liver samples from each of six cages of black, five cages of pseudoagouti, and five cages of yellow mice.

TABLE 6. Effect of Age and Phenotype on in Vitro Enzyme Activity (*Continued*)

Age (wk)			Treatment comparison[k]	
56[h]	86[h]	$\bar{P}$[i]	Age[e,j]	Phenotype[e,j]
0.90 ± 0.18	1.28 ± 0.13	1.17 ± 0.08	B: N.S.	8: N.S. 17: N.S.
0.81 ± 0.22	1.57 ± 0.18	1.31 ± 0.10	A: 17 > 8,30,56; 86 > 30	30: N.S. 56: N.S.
1.10 ± 0.24	1.68 ± 0.24	1.35 ± 0.09	Y: 86 > 56	86: N.S.
0.935 ± 0.121	1.51 ± 0.11		$\overline{W}$: 17,86 > 30,56	$\bar{P}$: N.S.
1.19 ± 0.27	1.69 ± 0.18	1.32 ± 0.10	B: N.S.	8: N.S. 17: N.S.
1.04 ± 0.27	1.99 ± 0.22	1.42 ± 0.11	A: 86,17 > 8,56; 86 > 30	30: N.S. 56: Y > B,A
1.88 ± 0.39	2.82 ± 0.42	1.87 ± 0.16	Y: 86 > All	86: Y > A,B
1.37 ± 0.19	2.17 ± 0.197		$\overline{W}$: 86 > All, 17 > 8	$\bar{P}$: Y > A,B
0.189 ± 0.059	0.304 ± 0.069		B: 17 > 56,8	8: N.S. 17: Y > B
0.074 ± 0.054	0.187 ± 0.016		A: 17,30,86 > 56; 17,30 > 86,8	30: Y,A > B 56: Y > A
0.272 ± 0.090	0.517 ± 0.146		Y: 30,17,86 > 56,8; 30 > 86	86: Y > A
			(Age × phenotype interaction)	
0.246 ± 0.079	0.391 ± 0.071		B: 17 > 8; 86 > 56	8: N.S. 17: Y > B
0.086 ± 0.059	0.237 ± 0.021		A: 17 > 86,8,56; 30 > 86,8,56	30: Y > A,B 56: Y > A
0.467 ± 0.155	0.847 ± 0.227		Y: All > 8; 30,17,86 > 56	86: Y > B,A
			(Age × phenotype interaction)	

[h] Data are derived from the 9000 × g supernatant of pooled liver samples from each of six cages of black, six cages of pseudoagouti, and six cages of yellow mice.

[i] Data are derived from the 9000 × g supernatant of pooled liver samples from each of six cages of black, six cages of pseudoagouti, and five cages of yellow mice.

[j] Mean phenotype value for all ages ± SE.

[k] Comparisons are significantly different at $p < 0.05$ by least square means in the general linear models procedure. When there were no age × phenotype interactions, $\overline{W}$ and $\bar{P}$ comparisons were tested at $p < 0.05$ by Duncan's multiple-range test.

[l] N.S. indicates the comparisons were not significantly different.

TABLE 7. Effect of Age and Phenotype on Hepatic Phospholipids[a]

Hepatic phospholipids	Phenotypes[b]	Age (wk)				Treatment comparison[g]	
		30[d]	56[e]	86[e]	P[f]	Age[b,h]	Phenotype[b,h]
Sphingomyelin	Black a/a	243 ± 12	247 ± 9	179 ± 7	220 ± 7	B: 56,30 > 86	30: N.S.
	Pseudoagouti A^{vy}/a	228 ± 10	230 ± 18	184 ± 17	216 ± 7	A: 56,30 > 86	56: N.S.
	Yellow A^{vy}/a	225 ± 4	233 ± 12	181 ± 10	216 ± 6	Y: 56,30 > 86	86: N.S.
	$\bar{W}$[c]	232 ± 6	237 ± 8	182 ± 7		$\bar{W}$: 56,30 > 86	$\bar{P}$: N.S.
Phosphatidylcholine	Black a/a	8876 ± 463	8858 ± 531	7295 ± 206	9039 ± 261	B: 30,56 > 86	30: N.S.
	Pseudoagouti A^{vy}/a	8589 ± 392	8319 ± 780	7700 ± 609	8809 ± 261	A: N.S.	56: N.S.
	Yellow A^{vy}/a	8956 ± 387	8575 ± 629	7124 ± 292	8771 ± 262	Y: 30,56 > 86	86: N.S.
	$\bar{W}$[c]	8798 ± 231	8584 ± 359	7373 ± 229		$\bar{W}$: 30,56 > 86	$\bar{P}$: N.S.
Combined phosphatidylserine and phosphatidylinositol	Black a/a	2033 ± 126	1838 ± 90	1432 ± 84	1886 ± 64	B: 30,56 > 86	30: N.S.
	Pseudoagouti A^{vy}/a	2003 ± 102	1717 ± 128	1469 ± 118	1882 ± 65	A: 30 > 86	56: N.S.
	Yellow A^{vy}/a	2022 ± 74	1771 ± 105	1365 ± 77	1845 ± 67	Y: 30,56 > 86	86: N.S.
	$\bar{W}$[c]	2019 ± 57	1775 ± 60	1422 ± 52		$\bar{W}$: 30,56 > 86 30 > 56	$\bar{P}$: N.S.
Phosphatidyl-ethanolamine	Black a/a	3460 ± 137	3803 ± 145	2829 ± 65	3638 ± 102	B: 56,30 > 86	30: N.S.
	Pseudoagouti A^{vy}/a	3585 ± 164	3464 ± 331	2902 ± 168	3558 ± 108	A: 30,56 > 86	56: N.S.
	Yellow A^{vy}/a	3342 ± 106	3568 ± 197	2765 ± 110	3465 ± 104	Y: 56,30 > 86	86: N.S.
	$\bar{W}$[c]	3469 ± 80	3612 ± 133	2832 ± 67		$\bar{W}$: 56,30 > 86	$\bar{P}$: N.S.
Total phospholipids	Black a/a	14612 ± 720	14746 ± 741	11735 ± 318	13698 ± 478	B: 56,30 > 86	30: N.S.
	Pseudoagouti A^{vy}/a	14404 ± 647	13730 ± 1248	12255 ± 900	13463 ± 566	A: N.S.	56: N.S.
	Yellow A^{vy}/a	14545 ± 373	14146 ± 914	11437 ± 458	13307 ± 496	Y: 30,56 > 86	86: N.S.
	$\bar{W}$[c]	14519 ± 338	14207 ± 547	11809 ± 342		$\bar{W}$: 30,56 > 86	$\bar{P}$: N.S.

[a] Values are mean μg phospholipid/g liver ± SE.

[b] Phenotypes: B, black *a/a* mice; A, pseudoagouti A^{vy}/a mice; Y, yellow A^{vy}/a mice.

[c] Mean age value for all phenotypes ± SE.

[d] Data are derived from the 9000 × g supernatant of pooled liver samples from each of six cages of black, six cages of pseudoagouti, and five cages of yellow mice.

[e] Data are derived from the 9000 × g supernatant of pooled liver samples from each of six cages of black, six cages of pseudoagouti, and six cages of yellow mice.

[f] Mean phenotype value for all ages ± SE.

[g] Comparisons are significantly different at $p < 0.05$ by least square means in the general linear models procedure. When there were no age × phenotype interactions, $\bar{W}$ and $\bar{P}$ comparisons were tested at $p < 0.05$ by Duncan's multiple-range test.

[h] N.S. indicates the comparisons were not significantly different.

tissue storage of lindane and its metabolites, (2) accelerated metabolism to 2,3,4,6-TCCOL concomitant with increasing age and elevated tissue storage of lindane and its metabolites, (3) higher dechlorinase activity, (4) reduced urinary excretion of lindane metabolites in the older animals, (5) elevated proportions of conjugated metabolites in the urine of older mice, (6) a decrease in hepatic phospholipids in the oldest mice, and (7) reduced glutathione-*S*-transferase activity compared to the lean siblings.

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